[1]林钟劝,陈瑶,韩梅,等.双循环线性滚环扩增检测HBV替诺福韦耐药位点的方法学研究[J].第三军医大学学报,2013,35(11):1093-1096.

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Title: Detection of tenofovir resistance gene of HBV by circular rolling circle amplification

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关键词: 双循环滚环扩增; 耐药位点; 突变

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摘要: 目的 探讨双循环线性滚环扩增（rolling circle amplification，RCA）检测HBV替诺福韦耐药基因突变位点的可行性。 方法 以HBV替诺福韦耐药基因突变位点为检测靶点，设计该位点的锁式探针以及包括该位点的HBV野生型、突变性模板。锁式探针与野生型模板特异识别并结合，通过*E.coli*连接酶连接为闭合环形结构，加入引物启动第1轮RCA；扩增产物用*Hpa* Ⅰ酶切游离出检测模板，重复上述过程进行第2轮RCA，琼脂糖凝胶电泳检测扩增产物；并对该方法的特异性、检测限及连接效率的影响因素进行初步探讨。 结果 探针与模板的杂交温度为45 ℃时，探针的环化连接效率最高、特异性最好。通过对不同浓度野生型模板的检测，单循环RCA的检测限为50 pmol/L，双循环RCA的检测限为5 pmol/L，检测灵敏度提高了10倍。 结论 初步建立了检测HBV替诺福韦基因耐药位点突变的双循环RCA方法。

Abstract: Objective To investigate the feasibility of using circular rolling circle amplification (RCA) to detect the resistance gene to tenofovir in hepatitis B virus (HBV). Methods The mutation site of resistance gene of HBV to tenofovir was taken as target to design the padlock probe. Wild and mutation templates were also synthesized in this research. The designed padlock probes were linked to closed rings by *E.coli* DNA ligase when they specifically bonded to wild templates. RCA was started when primers annealed to the probes. Then *Hpa*

I cut off the product drastically, and more templates were released. Then the processes above were repeated to start the RCA for a second time. Finally the product was detected by 1% agarose electrophoresis. The influential factors of ligation efficiency, specificity and sensitivity were also studied. Results The ligation efficiency and specificity of the probe were enhanced when the hybridization temperature was set at 45 ℃. Among the different concentrations of wild template detected, the lowest rate of RCA was 50 pmol/L, and it was as lower as 5 pmol/L when the circular RCA was used. Conclusion Circular RCA is established successfully to detect resistance gene of HBV to tenofovir.

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